Effect of Shade on Seed Germination, Growth and Xylem Development of *Moringa oleifera* Seedlings

By

Lamia Tewfik Ahmed Mohamed

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Dr. Essam Eldin Ibrahim Warrag

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بسم الله الرحمن الرحيم

قال سبحانه وتعالي:

(وفوق كل ذي علم عليم)

"صدق الله العظيم"

Dedication

TO

Soul of my mother

My lovely husband Wally

My honey daughters Leen and Layan,

My parents

My brothers and sisters

My relatives and friends

For everybody who knows who I am

I dedicate this study

With my love

Lamia

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Abstract

Title: Effect of Shade on Seed Germination, Growth and Xylem Development of *Moringa oleifera* Seedlings (Alrawag seedlings)

Lamia Tewfik Ahmed Mohamed

This study investigated the effect of shade as a moderator of light intensity and temperature on germination percentage and germination rate, biomass accumulation and partitioning and xylem development of seedlings of *Moringa oleifera*.

Three levels of shade were studied: high shade (80%), medium shade (50%) and no shade (0%). Germination rate and germination percentage were calculated after two weeks from seed sowing. Four sequential harvests were carried out starting from four weeks after seed sowing. Seedling growth parameters (shoot length, root length, shoot dry mass, root dry mass, total dry mass and root/shoot ratio of the dry mass) were assessed at each harvest. Cross sections were prepared from the lower and middle parts of the stems at each harvest and anatomical parameters were measured (vessel volume fraction, vessel diameters, number of vessel and volume fraction of lignified cells).

Result showed that the effect of shade was significant on germination, seedling growth and anatomical parameters. The medium shade (50%) had significantly fast rate of germination than no shade and higher germination percentage than high shade. Generally, the medium shade

(50%) gave higher values for the seedling growth parameters and produced erect and strong shoot than the other treatments. The values of the anatomical parameters of the stem wood of the seedlings decreased with the increase in shade level.

These findings indicate the importance of shade to the germination and seedling development of *M. oleifera* and that should be considered in the nursery conditions. The study recommends that medium shade level (50%) to be used for seedling production.

مستخلص البحث

العنوان : تأثير الظل على إنبات البذور و نمو و تطور خشب شتول المورنقا (شتول الرواق)

لمياء توفيق احمد محمد

استهدفت الدراسة اختبار تأثير الظل كوسيط بين شدة الإضاءة ودرجة الحرارة على نسبة الإنبات ومعدل الإنبات ؛ توزيع و تراكم الكتلة الحية وتطور الخشب في شتول المورنقا.

تمت دراسة ثلاثة مستويات من الظل وهى ظل عالي (80%)، ظل متوسط (50%) ولا ظل (0%). تم حساب كلاً من معدل الإنبات و نسبة الإنبات بعد إسبوعين من الزراعة. أجريت أربع حصدات متتابعات ابتدا من اربع اسابيع من الزراعة. قدرت فى كل حصدة متغيرات النمو للشتول (الطول الخضري، الطول الجذري، الكتلة الجافة الخضرية، الكلتة الجافة الجذرية، الكتلة الجافة الكلية و نسبة الكتلة الجافة الجذرية/الخضرية). جهزت قطاعات عرضية من وسط وأسفل السيقان، أستخدمت تقنية العد الإسترولوجي لقياس المتغيرات التشريحية (نسبة الحجم للأوعية ، أقطارالأوعية، عدد الأوعية ونسبة حجم الخلايا المتلجننة).

اظهرت النتائج وجود تاثيرمعنوى للظل على الإنبات، نمو الشتول والمتغيرات التشريحية. الظل المتوسط (50%) كان لديه معنوياً اسرع معدل انبات مقارنةً باللاظل، واعلى نسبة انبات مقارنةً بالظل العالى. عموماً، الظل المتوسط أعطى اعلى قيمة لمتغيرات نمو الشتول وانتج مجموع خضرى قائم وقوى مقارنة بالمعاملات الاخرى. انحفضت قيم المتغيرات التشريحية لخشب الساق فى الشتول مع زيادة مستوى الظل. أشارت النتائج أن اهمية الظل لانبات وتطور المورنقا يجب ان يعطى اعتباراً فى ظروف المشتل. أوصت الدراسة بأستخدام مستوى الظل المتوسط (50%) لانتاج الشتول.

CHAPTER ONE
INTRODUCTION

1.1 Back ground

Moringa oleifera (Moringa), is a well known and cultivated species of the thirteen species of the family *Moringacae* and it is one of the world's most useful plants (Fahey, 2005). It is native to the sub-Himalayan tracts of India and it has become naturalized in many locations in the tropics and is widely planted in Africa (Fahey, 2005). It is known by several names but is popularly called the "drumstick tree" for its fruits that are used by drummers and the "horseradish tree" for the flavor of its roots (Palada and Chang, 2003).

The species is drought resistant, though in drought conditions it may lose its leaves, and it recover when it rains (Von Mydell 1986). It grows well in areas receiving annual rainfall amounts that range from 250 to 1500 mm (Amaglo, 2006). It survives in a temperature range of 25°c to 40°c but has been known to tolerate temperature of 48°c and light frosts. It prefers neutral to slightly acidic soil and grows best in well-drained loam to clay-loam and it tolerates clay soils but does not grow well if waterlogged (Von Mydell, 1986).

The species have multiple uses and that have attracted the attention of researchers, development workers, and farmers. It is

becoming a vital source of nutrition, where most of the world's poor people live (Palada and Chang, 2003).

M. oleifra is one of the important tree species, and widely distributed in Sudan (Elamin, 1981). It was originally an ornamental tree planted during British rule. Because of its many uses, it is planted in the whole tropical belt and in the Sudan concerned it was planted as "clarifier tree" (Shagarat Al rawag) (Dishna, 2000).

1.2 Justification

The growth of most of the forest tree seedlings are affected in the early stage by moisture, sun light intensity, temperature, and other factors (Bonner, 1984). *M. oleifera* can be cultivated very cheaply at the household level or in small communal nursery which is to be encouraged among the rural population (Dishna, 2000). However, the effect of external environmental factor on seedlings growth is not fully studied under dry condition in Sudan. Especially the effect of light intensity and temperature, which in turn affected by shade. Despite the valuable uses of *M. oleifera* very little is known about its response to light intensity in Sudan. In nursery practice, it is important to obtain the suitable light conditions to produce well balanced and hardened seedlings suitable for out planting.

Diversity of form is a characteristic shared by many plant groups in dry tropical habitats, which are thought to support the world's highest diversity of plant life forms (Medina, 1995). However, little is known about the anatomical correlations of the

great diversity seen at the morphological level in dry tropical plants (Olson and Carlquist, 2000).

Accordingly, the studies dealing with the effect of shade on the vegetative character and wood formation on the *M. oleifera* seedlings are important. This may lead to great changes in seedlings growth development in the nursery and further in the field.

1.3 Problem Statement

Jahn *et al*. (1986) reported that germination and growth of *M. oleifera* seedlings is much affected by light condition and the optimum light condition for germination of *Moringa species* was half shade. They indicated that the speed of germination of untreated seeds depended on temperature, humidity and watering. Further they reported that all seedlings from *Moringa species* grew poorly and succumbed at an early stage of development for unknown reasons.

Mohamad (1986) mentioned that seedlings grown at 30% and 50% light intensity have tall and slim shoots with large dark green leaves and relatively poor root system. These seedlings are unhardened and succulent and are generally sensitive to desiccation.

The observations indicate that *M. olifera* seedlings are affected by shade; high shade produces succulent seedlings (Figure 1), but it needs some shade in early stage.

Figure 1: Seedlings of *M. olifera* with succulent stems, under high shade conditions.

1.4 Objectives

The main objective was to study the effect of shade as a moderator of light intensity and temperature on germination, seedling growth and stem wood anatomy of *M. oleifera*.

The specific objectives were to study the effect of shade on:

1. Germination percent and rate.
2. An accumulation and partition of seedlings biomass.
3. Xylem development of seedlings.

CHAPTER TWO
LITERATURE REVIEW

2.1 *Moringa oleifera* Lam.

M. oleifera, is commonly referred as "Moringa" from Tamil language (Muringa**)** and Malayalam language (Murunggi). Is the most widely cultivated species of the genus *Moringa*, which is the only genus in the family *Moringaceae*. The English name of the species is Horse radish tree and the Arabic name is Rawag (Elamin, 1981).

2.1.1 Description

Gibreel (2008) described that *M. olifera* as small fast-growing, drought tolerant and deciduous tree that ranges in height from 5-12 m, with an open, umbrella shaped crown, straight trunk 10-30 cm thick. The bark is grey smooth, corky, roots of young plants swollen. Leaves are evergreen foliage (depending on climate) has leaflets 1-2 cm in diameter, bi or more often tripinnate; terminal leaflets obovate, and slightly larger than lateral one, generally with 6 pairs of pinnae, with 2 pairs of opposite laterals and one terminal. Flowres are in along branches, paniculate, sweet scented, cream coloured; sepals 5 unequal in size; petals unequal and slightly larger than sepals, white with yellow dots at the base. Fruits are capsules, 3-angled, elongate-linear tapering at both ends, 9-ribbed, up to 30 cm long; seeds round, with 3 papery wings. Flowering November-January; Fruiting takes place from January to March (Figure 2).

Figure 2: Typical *Moring olifera* trees carrying ripped fruits.

2.1.2 Distribution

Native to India, Arabia, and possibly Africa and the East Indies; widely cultivated and naturalized in tropical Africa, tropical America, Sri Lanka, Mexico, Malaysia and the Philippine Islands (Palada and Chang, 2003). The species was introduced to the Sudan and distributed widely in short grass savanna areas and also planted in many parts of the country (Elamin, 1990).

Originally considered a tree of hot, semiarid regions (annual rainfall 250–1500 mm) it has also been found to be well adapted to hot, humid, wet conditions (Dishna, 2000).

2.1.3 Cultivation

M. oleifera can be easily established by cutting or by seed. Seeds can be sown either directly or in containers and no seed

treatment is required (Dishna, 2000). Seeds can be planted as soon as they are mature but should only be kept for up to 3 months in natural conditions and before sowing the seeds can be soaked in water for one day and then planted (Von Mydell, 1986).

However it does best where temperature ranges from 26 to 40°C and annual total rainfall at least 500 mm. It grows well from sea level to 1000m in elevation (Dishna, 2000). It is wide range of adaptability is also shown in the variety of soil conditions where it grows continuously under water stress in 2cm apart and 1cm deep with frequent watering seeds (Dishna, 2000).

Von Mydell (1986) reported that; the propagation of cuttings from healthy branches with hard wood, 45cm to 1.5m long and 10cm wide is possible. The cuttings should be taken in the rainy season. The green wood should be trimmed without damaging the bark of the hardwood with leaving the cutting ends in a shady place for 3 days to dry. The cutting should be planted directly in the soil or in polythene bags containing 3 parts soil and 2 parts sand. One third of the cutting's length should be placed in the soil. The soil should be moist but not over watered. Cuttings planted in polythene bags will take a long time to develop roots and may be planted out after 2 or 3 months.

The Plants rose from 1m cutting beat pods from the second year and growth onwards with maximum production at 4 to 5 years. In a favorable environment an individual tree can yield 50 to 70 kg of pods in one year (Dishna, 2000).

2.1.4 Uses

Fahey (2005) indicated the importance of this species as a multi-function plant, it has been cultivated in tropical regions all over the world for the following characteristic: 1) high protein, vitamins, mineral and carbohydrate content of entire plants; high value of nutrition for both humans and livestock; 2) high oil content (42%) of the seed which is edible, and with medicinal uses; 3) The coagulant of seeds could be used for wastewater treatment. This plant has been well documented for its medicinal importance for a long time.

The leaves have more beta-carotene than carrots, more protein than peas, more vitamin C than oranges, more calcium than milk, more potassium than bananas, and more iron than spinach (Appendix 1) (Palada and Chang 2003). Also, the leaves are considered to offer great potential for those who are nutritionally at risk and may be regarded as a protein and calcium supplement, it is particularly useful as a human food in tropical countries because the leaves appear towards the end of the dry season when few other sources of green leafy vegetables are available and the powder of dried leaves can be added to sauces at the same time as other condiments or vegetables are added (Fahey, 2005).

The seeds are used to clarify water (rawag) due to their co-agulating properties in the northern Sudan, village women had so far used seed to treat the highly turbid water of the Nile (Jahn *et al.* 1986). The fruits and seeds are tastier while they are young and

before they turn brown. In Malaysia, the young tender fruits are cut into small pieces and added to curries (Fahey, 2005).

The root can be applied externally as a poultice in cases of inflammation, as a valuable rubefacient, it is also used as a substitute for horseradish. The effect of oral application of aqueous and alcoholic extracts of root-wood has been studied in male Wistar albino rats. Ethylene glycol feeding resulted in hyperoxaluria as well as increased renal excretion of calcium and phosphate. Supplementation with aqueous and alcoholic extracts of M. oleifera root-wood significantly reduced the elevated urinary oxalate, showing a regulatory action on endogenous oxalate synthesis (Fahey, 2005).

The increased deposition of stone forming constituents in the kidneys of calculogenic rats was also significantly lowered by curative and preventive treatment using aqueous and alcoholic extracts. The results indicate that the root-wood of *M. oleifera* is endowed with antiurolithiatic activity (Fahey, 2005).

The wood provides a pulp that is considered suitable for paper, wrapping, textiles and cellophane. According to Verma *et al.* (1976) the species had being planted in India on large scale as a potential source of wood for the paper industry. In Jamaica, exudates are used for blue dye (Fahey, 2005).

The different parts of the plants were used in folk medicine as flowers, leaves, and roots are used in folk remedies for tumors, the seed for abdominal tumors. The root decoction is used in Nicaragua for dropsy. Root juice is applied externally as rubefacient or counter-

irritant and the leaves applied as poultice to sores, rubbed on the temples for headaches, and said to have purgative properties (Hartwell, 1971).

Bark, leaves and roots are acrid and pungent, and are taken to promote digestion. Oil is somewhat dangerous if taken internally, but it can be applied externally for skin diseases. Also, the bark regarded as antiscorbic, and exudes a reddish gum with properties of tragacanth; sometimes used for diarrhea. Roots are bitter and act as a tonic to the body and lungs, and are emmenagogue, expectorant, mild diuretic and stimulant in paralytic afflictions, epilepsy and hysteria (Hartwell, 1971).

2.2 Germination

Germination is defined as "the resumption of active growth in an embryo which results in its emergence from the seed and development of those structures essential to plant development" (Bonner, 1984). In another sense, it is the culminating event of seed maturation, the establishment of the seedling. It can be viewed as occurring in overlapping events: Absorption of water; Increased respiration, enzymatic activity, and assimilation of stored foods; Increased adenosine phosphate and nucleic acids; Cell growth and division; and Differentiation of tissues (Kramer and Kozlowski, 1979). All of these events are influenced by environmental conditions and events within the seeds themselves (Paulo, 2006).

2.2.1 Germination rate

This characteristic is important to predict the degree of success of a species based on the capacity of their harvest seed to spread the

germination through time, permitting the recruitment in the environment of some part of the seedlings formed (Paulo, 2006).

2.2.2 Factors affecting seed germination

Bonner (1984) found that the most important environmental factors that influence germination are: moisture, temperature, light, and aeration.

Moisture: The typical pattern of moisture uptake by seeds has 3 phases (Vertucci, 1989): a rapid initial uptake, a short lag period of extremely slow uptake, and another rapid period of uptake just before germination. The first phase is primarily imbibitional in nature and occurs in dead seeds as well as live ones (Simon, 1984). It is a physical process of moisture moving from a substance with high water potential (soil) to one with a low water potential (dry seed) (Simon 1984). This uptake displaces gases from dry seeds, and is visually evident in the bubbles that slowly escape from dry seeds when they are submerged in water (Simon, 1984). The length of the second phase is related to the degree of dormancy or delayed germination in the seeds (Simon, 1984). It can be practically absent in the rapidly germinating seeds or extended in the case of very dormant seeds. The third phase occurs when metabolism becomes very active, and the seed coats split, leading to greater oxygen uptake (Bonner, 1968).

Temperature: Seeds of temperate woody plants can germinate over a wide range of temperatures, from a minimum of 2 or 3 °C, to a maximum of about 45 °C. Radical emergence occurs in most species at 45 °C, but few produce normal seedlings at this temperature. Low

temperatures, on the other hand, are favored by some species (Bonner *et al*., 1994).

Light: Light plays a complex role in the germination of woody plants. It stimulates the germination of most species but is absolutely necessary for only a few. It is often difficult to separate the effects of light and the effects of temperature. Dry, dormant seeds normally do not germinate in the dark, but stratification at low temperatures or treatment with high temperatures can overcome the dark inhibition in some species (Bonner and Vozzo, 1987).

2.3 The effect of shade on propagation

When water and nutrients are not limiting, reduction in light is the most important environmental constraint arising when plants are cultivated in dense plantings. In particular, the assimilation and redistribution of carbon are greatly affected by plant density. It has been clearly established that, at high densities, competition for light changes the relationships between the aerial part and the root system, together with the morphology (size, architecture) of the plant. Under light-limited conditions, the growth of roots is reduced more than the growth of the aerial parts, which leads to a decrease in the root/shoot ratio (Pellerin and Demotes-Mainard, 1992).

In addition, under light competition the root/shoot biomass ratio decreased with growth. Also, shaded isolated plants behave as plants under increased density conditions. Shoot biomass accumulation is less inhibited than root biomass accumulation by light competition, compared with full illumination (Hébert *et al.,* 2001).

Mohamad (1986) reported that seedlings of *Shorea materialzs* require moderate shade for their highest growth. The best height growth occured at a light value of 55% relative light intensity (RLI). The ratio of shoot weight to root weight is one of the important indicators of the condition of nursery stock. Strong sunlight reduces shoot growth, and slightly promotes root growth. Hence the ratio of shoot weight to root weight is higher in a shade condition for *Shorea materialis* seedlings.

Roots are of many sizes and shapes and shoot⁄ root biomass ratio in some species may change dramatically during the life cycle of the plant. The shoot⁄ root relationships are influenced by genetics and the environmental combinations that exist during plant growth. Comparison of genetically- identical seedlings under different growth environments show that the plants with the largest shoot may or may not have the largest root system, depending on environmental conditions (Kasperbauer and Hunt, 1992).

The ″strategy" of each plant is to sense the total growth environment, and invest only enough carbon in roots to support the plant as it proceeds its life cycle. Excessive investment of carbon in a root system might be at the expense of photosynthetic area and affect seed yield per plant. Some of the environment variables that influence the relative size of shoots and roots include: photoperiod (day length), moisture stress, soil acidity, nutrient availability, shoot and root temperatures, diseases, insects, and plant population density

which affects intensity and spectral distribution of light received by the growing plants (Kasperbauer and Hunt, 1992).

Compared with seedlings grown at a high light intensity, seedlings grown at 30% and 50%RLI have tall and slim shoots with large dark green leaves and relatively poor root system. These seedlings are unhardened and succulent and are generally sensitive to desiccation. Therefore, in nursery practice, it is important to maintain more open light conditions to produce well balanced hardened seedlings suitable for out planting (Mohamad, 1986).

Jahn *et al.* (1986) mentioned that the speed of germination of untreated seeds of *M. oleifera* depended on temperature, humidity and watering; the optimum light condition for germination of all Moringa species was half shade.

Also, Jahn *et al.* (1986) indicated that exposure to full light did not greatly affect the germination of *M. oleifera* seeds that had been sown during the cool dry season. After sowing in the hotter weather in mid-April, however, germination frequencies amounted to only 40 and 52 percent in full light as compared with 94 and 92 percent in half shade.

As with germination, growth of the seedlings is much affected by light conditions, particularly during the hot periods of the year (Jahn *et al.*, 1986; GFU, 2008). Early removal of tender seedlings to full light, combined with irregular watering, can thus have disastrous consequences; the average and maximum heights of *M. oleifera* seedlings were 1.7-2.2 times higher in half shade than in full light.

Within each species, those seedlings appearing first in a batch usually exhibited fastest development (Jahn *et al.*, 1986).

In addition, Jahn *et al.* (1986) indicated that growth of seedlings in half shade takes place more slowly in the hot dry season than in the cool dry season. To obtain stable healthy seedlings, the seeds should therefore be sown either during the rainy season or during the dry, season. Moreover, there is evidence that the seedlings should not be transplanted too early.

2.4 Wood structure

Hardwoods structure is more complex in comparison to softwoods. Hardwoods are composed of at least four major kinds of cell namely, vessel elements, fibers, rays and parenchyma cells. Each of these may constitute 15% or more of the volume of hardwood xylem (Haygreen and Bowyer, 1980). Mahjoub (2001) mentioned that the hardwoods elements differ in their proportion and arrangement from one species to another and within the individual tree.

2.4.1Vessels

Vessel elements are generally much shorter and larger in diameter than other types of longitudinal cells. The short length of vessel elements is traceable to the fact that they often do not grow in length during the maturation process and may become even shorter than the cambial initials from which they were produced (Jane *et al.*, 1970). Vessels are composite tube- like structures of indeterminate length. They are made up of individual cell called vessel element which are fused together end to end with complete or partial

disappearance of end walls. The pores formed in the early wood are much larger and more crowded than those formed in the latewood (Core *et al.,* 1976). The appearance based on the orientation in which vessel are grouped as seen in transverse section, has been used for the recognition of vessel types (Carlquist, 1984).

The term vessel clusters is applied to vessel groupings in which vessels touching each other form a collection about as wide tangentially as radially (Carlquist, 1988).The term aggregation is used for those groupings that are more extensive and often extend across rays as seen in wood transverse sections (Carlquest, 1987). Diagonal aggregation (grouping often traversing rays) that oriented in directions midway between radial and tangential are considered "are-porous" (Kukachka, 1978). Tangential aggregation is the tangential band of vessels that represents not a single category, but several phenomena. In one of these there are large non-grouped early wood vessels followed by tangential bands of smaller vessels (Carlquist, 1987).

Vessels as seen in transverse section may be a few to many, and this is commonly recorded in the number of vessels seen per mm^2 of transverse section. Number above 500 is unusual, but has been found in plants of notably dry habitats (Michener, 1983; Carlquist and Hoekman, 1985) or notably cold habitats (Miller *et al*., 1975).

2.4.2 Variability in structural characteristics of wood

The environment acting on a tree is complex and constantly changing and the response of the tree to the environment is equally complex and similarly changing. Two major factors rule the common trends of variability in wood. Those are genetics and environmental influence.

Fielding and Brown (1960) defined the gross heritability to be the proportion of the total variance of characteristic which is due to hereditary differences among individuals. The earliest most conclusive proof of genetic influence on cell length of hardwoods has been obtained from the discovery that natural papules triploids have superior fiber length to normal diploids of about 21-26 percent.

Successful rising of forest plantations would largely depend upon the selection of appropriate tree species which can thrive well in degraded and harsh soil with extremes of moisture supply. Plants, by nature, possess remarkable adaptive mechanisms to avoid or tolerate drought stress (Levitt, 1972).

The most dramatic changes observed among the anatomical parameters considered herein were in the development of the secondary xylem and sclerification with stem development, as well as in the etiolation and shading effects on lignifications and sclereid formation (Maynard and Bassuk, 1996). Rooting percentages were positively correlated with changes reflecting increased succulence of the stem, e.g., a thicker cortex, less lignifications of the secondary xylem, thinner periderm and a higher percentage of sclereid- free

gaps in the perivascular sclerenchyma. This succulence could be attributed to either the stock plant treatments of etiolation or shading ,or to the extent of shoot development (Maynard and Bassuk, 1996).

2.4.3 Vessels adaptation

Xylem adaptation to various growth conditions has been studied in different ways. In a group such as Asteraceae (which have libriform fibers) degree of vessel grouping rises markedly in relation to dryness of the habitat Vessel element tends to increase with increase in diameter; growth rings show clearly the independence of the two dimensions. Vessel element diameter and length decrease with aridity, but it may be that if air bubbles can be localized within individual vessel elements, even those with simple perforation plates, shorter vessel elements would be adaptive in more arid situation (Carlquist and Hoekman, 1985).

Longer vessel elements have been shown to be correlated with more mesic habitats. The correlations between vessel element length and altitude or latitude should be traced to factors of water availability and temperature. Since altitude and latitude, are not ecological factors in themselves (Baas 1973).

Vessel dimensions are sensitively related to ecology, while there is definitely a heritable component. There is wide latitude for phenotypic modifiability also as can be demonstrated where a given genetic stock is grown in two or more different localities (Bissing, 1976; Akahchuku and Burley, 1979).

Doley (1978) reported that seedlings of *Eucalyptus grandis* were grown under light regimes for 7 weeks, during which time secondary xylem development was studied. The rate of cambial cell division was approximately 50% greater in the light than in the low light treatment.

For many genera and species, diameter and vessel element length decrease while vessel frequency increases with decreasing water availability (Baas and Schweingruber, 1987; Van der Walt *et al*., 1988; Zhang *et al*., 1988; Wilkins and Papassotiriou, 1989; February, 1993). Thus, a xylem with narrow vessels is physiologically better protected against cavition (Rury and Dichinson, 1984).

2.4.4 STEREOLOGY

Stereology is the body of methods for the exploration of three-dimensional space, when only two-dimensional sections through solid bodies or their projections on a surface are available (Elias, 1976). Stereological methods were used to evaluate different characterization and to avoid the disadvantage of the manual methods which are time-consuming, laborious and have the probability of increasing error (Nasroun and Elzaki, 1987). Stereology principles are used in metallurgy, mineralogy and geology for studying micro-structural properties that are related to physical and mechanical properties of materials (Nasroun, 1978).

Certain basic measurements are required repeatedly in all quantitative stereology works. These basic operations are preformed

on two-dimensional sections or projections, and involve simple point's counts, intersection counts and number of features (Nasroun, 1979). From these counts the following parameters were calculated: point count (Pp), the number of points of intersection with boundaries generated per unit length of test lines (PL) and the number of objects or feature in vessels in a contained area of microstructure (NA).

1- Pp (point counting) is one of the simplest operations of stereology. The term refers to the number of test points falling on a particular structure divided by total number of test points. These test points could be intersection of test lines making the test grid or end points of short test lines or random points on a grid (Ifju, 1983).

The stereological equations that relate the average point fraction (PP), lineal (surface) fraction (LL), area fraction (AA) and volume fraction (VV) is given by:

PP = AA = LL = VV (Ifju 1983).

Where PP is the average of several randomly applied point, LL is the average of lineal fractions, AA is the fraction of the total area of a section which is occupied by the element and VV is the volume fraction which is the volume occupied by structural feature per unit volume of the structure (Underwood, 1970).

2- PL is the number of intersection with boundaries generated per unit length of test lines (Ifju, 1983). The procedure involves superposition directed line segments upon the microscopic section images. Account of the number of the times that the line segment intersects the cell boundary when divided by the actual segment

length will give the number of intersections per unit. A linear or circular test array is applied randomly or placed systematically over the entire microstructure until a sufficient number of intersections have been counted. The actual total grid length (L) depends on the magnification of the microstructure, but its value can be determined at standard magnification (Nasroun, 1979).

3- NA is the number of objects or features in a certain area of microstructure; it allows the average area (A) of the cells to be calculated using the following equation:

A = AA / NA = The point counting (PP) / NA (Ifju, 1983).

The above count for applying stereological equations for estimating the dimensions and proportions of anatomical features.

CHAPTER THREE

MATERIALS and METHODS

3.1 Site preparation and seed source

The study was conducted in a site near the nursery of the Faculty of Forestry, University of Khartoum. The site was cleared of bushes and the soil was leveled, and then was divided into three square units (1.5m x 1.5m). Each unit was then assigned randomly to one of the three studied treatments, which were high, medium and no shade. Four pillars were erected in the four corners of each unit and then covered with green nets on the top and the four sides to give the required shade. One green net layer was erected for medium shade, double green net layer for high shade and no green net layer for no shade. Aeration openings were made in each unit on the northern and southern sides of treats.

Fruits of *M. oelifera* were collected from trees in the study site (near the nursery). Seeds were then extracted from the Fruits (Plate 3), with an average weight of 0.232g per seed; the seeds were examined on dishes in the laboratory; and the germination percentage was 85%.

Figure 3: Fruits and seeds of *Moringa oleifera*

3.2 Treatment and design:

The treatments consist of high shade (HS, 80% shade), medium shade (MS, 50% shade), and no shade (NS, 0% shade). The respective shades were obtained using green net layers. One green net layer was erected for medium shade, double green net layer for high shade and no green net layer for no shade.

In each unite a total of 100 polythene bags (20*10cm) were filled with clay soil (2.75kg), two seeds were randomly planted in each polythene bags and total of the seeds used were 200. The sown seeds were irrigated every other day by surface irrigation.

3.3 Germination count:

Germination was counted daily for two weeks and the following was calculated:

1. Days for 50% germination (50% of total seeds were germinated) (germination speed) were determined as following: (N1D1 + N2D2 +...+ N50%D50%), where D1, D2,..., Dx: D1, the first day of germination; D2, the second day of germination and so on until the last day, when half of total seeds had germinated D50%. Also, N1, N2,..., N50%: N1, number of seeds had germinated on the first day, N2, number of seeds had germinated on the second day, and so on until the last day, when half of total seeds were germinated N50%.

2. Germination rate (germination speed) was determined as following: (N1D1 + N2D2 +...+ NxDx)/ (N1 + N2 +...+ Nx) 100, where D1, D2,..., Dx: D1, the first day of germination; D2, the second day of germination and so on until the last day of germination Dx. Also, N1, N2,..., Nx: N1, number of seeds had germinated on the first day; N2, number of seeds had germinated on second day and so on until the last day Nx.

3. Germination percent: calculated as number of germinating seeds after 15 days to total number of seeds times100 (eg :90/100*100=90%).

After germination, seedlings were singled out randomly and one seedling was left per bag.

3.4 Seedling harvest:

The first harvest was carried out after 4 weeks from seed sowing followed by second harvest after 8 weeks, third harvest after 12 weeks, and the fourth harvest after 16 weeks. From each treatment 25 seedlings were harvested, and then 20 seedlings were assigned randomly to measure growth variables and the remaining 5 seedlings were used for stem anatomy variables.

3.4.1 Seedling growth variables:

The following variables were determined at each harvest.

- Shoot length (cm).
- Root length (cm).
- Shoot dry mass (g).
- Root dry mass (g).
- Total dry mass (g).
- Shoot dry mass to Root dry mass ratio.
- Stem strength - stem resistance to cut:

This last measure was done only for seedlings at harvest three. It was measured by breaking the stem manually and scored as soft or hard. Also, Stem resistance to cut was checked by cutting with razor and scored as easy to cut, hard to cut and very hard to cut.

3.4.2 Stem wood anatomy:

3.4.2.1 Slide preparation:

Five seedlings were randomly chosen out of the 25 seedlings of each treatment to prepare microscope slides. Cross sections were prepared from the lower and middle parts of the stems. The sections were put in Petri-dish filled with water and examined under the microscope by mounting in a drop of water on clean slide to select good quality sections to be stained and mounted.

Sections were put in 50% alcohol for one minute, then transferred to Safranin (0.2 g/100 ml 50% alcohol), left for 10 minutes (to be fixed at the lignified cell walls) and then washed in 50%, 70% and 96% alcohol. The sections were then transferred to light green stain (0.2 g/100ml alcohol 95%) for one minute, washed in 96% alcohol and then in absolute alcohol and finally transferred to xylol. Each section was mounted in a drop of Canada balsam on a clean slide, then covered with a cover slip and labeled.

3.4.2.2 Microscopic examination

Photographs of the slides for the stem wood cross-sections were taken using a 'Moticam 1000' digital camera mounted on an 'Olympus CH20' microscope using a 10×10 magnification (Appendix 2). The images were printed on A4 paper.

3.4.2.3 Stereology count techniques

Stereological count was conducted following the procedure of Ifju (1983). A sixteen-point grid (3×3 cm in dimension) was drawn on a paper and then it was photocopied on a transparent paper (Plate 4). The grid was fixed on each of the photographs to obtain the point counting (Pp), the number of points of intersection with boundaries generated per unit length of test lines (PL) and the number of objects or feature in vessels in a contained area of microstructure (NA). A glass scale was projected through the microscope and the calibration was made to find the total stereo logical area.

For each shade treatment, 5 slides (one from each of the 5 sample seedlings) were used and four fields from each slide were examined. The stereological data were entered in the computer and calculations were made to determine the following anatomical features using Equations 3-1, 3-2 and 3-3 (Ifju, 1983)

- Vessel volume fraction.
- Vessel diameter.
- Number of Vessel per /mm^2.
- Volume fraction of lignified cells.

Volume fraction for vessel was determined as following:

$$\mathbf{CF} = \mathbf{PP}_L + \mathbf{PP}_W \quad \text{..... 3-1}$$

Where:

CF = Volume fraction of vessel.

PP$_L$ = Volume fraction of vessel lumen.

PP$_W$ = Volume fraction of vessel cell-wall.

Horizontal cell diameter of vessel elements was determined as following:

$$\textbf{CD}\ (H) = \frac{P_L}{2N_A} \quad \text{..... 3-2}$$

Where:

CD (H) = horizontal cell diameter of vessel elements.

P$_L$ = the number of intersection points (4*4) of the four horizontal lines of test grid with boundaries of vessels lumen area.

N$_A$ = the total number of objects vessels which exist in the total area of the grid.

$$\textbf{CD}\ (V) = \frac{P_L}{2N_A} \quad \text{..... 3-3}$$

Where:

CD (V) = vertical cell diameter of vessel elements.

P$_L$ = the number of intersection points of the four vertical lines of test grid with boundaries of vessels lumen area.

N$_A$ = the total number of objects vessels which exist in the total area of the grid (4*4).

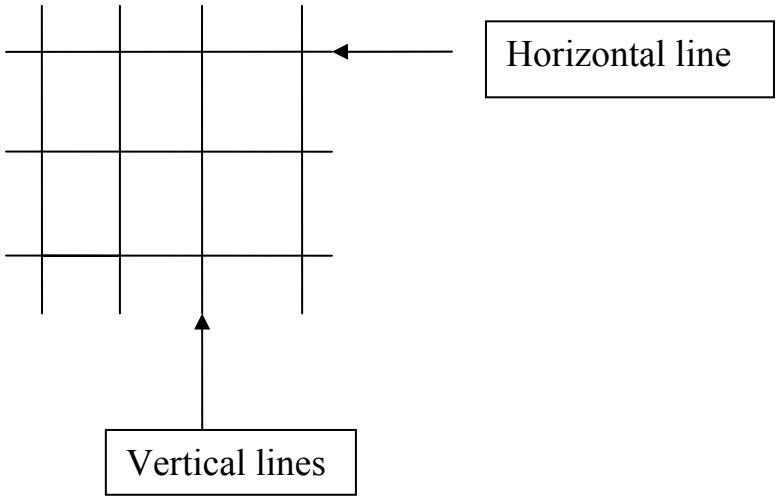


Figure 4: **The stereology area (the grid).**

3.5 Data analysis

The data of germination, seedlings growth and stem wood anatomical variables of *M. oliefera* were recorded and analyzed for the three levels of shade.

One way analyses of variance was conducted to study the effect of the shade on each of the growth and anatomical variables. Treatment means were separated using Duncan's Multiple Range Test when the effect of the treatment was significant. Statistical analyses were conducted using SAS statistical package (SAS Institute, 1995).

CHAPTER FOUR

RESULTS

4.1 Effect of Shade on Germination

The shade level had high significant effect on the studied germination variables after two weeks from date of sowing ($p \leq 0.001$). Significant differences were observed between the means of the three tested levels of shade (Table 1, Figure 1).

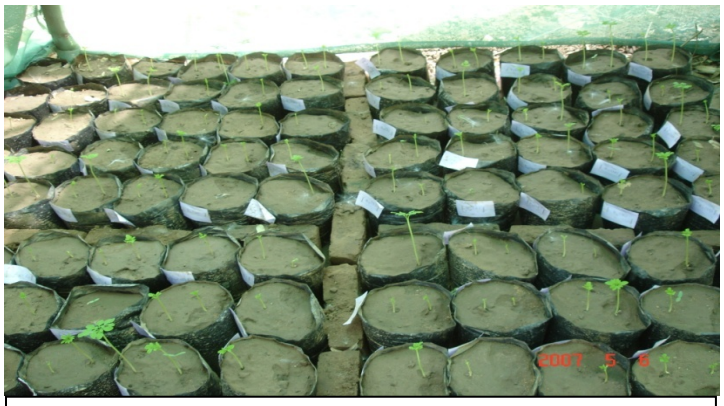
The effect of shade on days for 50% germination and germination rate was highly significant ($P = 0.0001$). The days for 50% germination and germination rate of the high and medium shades had significantly lower values than no shade (Table 1).

The effect of shade on total germination percent after two weeks was highly significant ($p= 0.0012$). The percentages of the medium and no shades were significantly higher than the high shade.

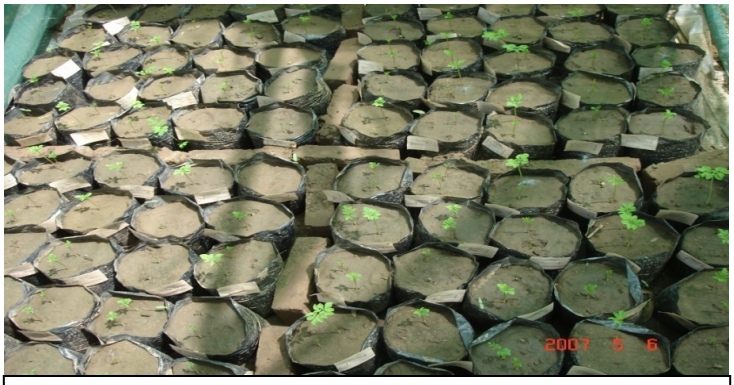
Table 1: Effect of three shade levels on germination variables of *Moringa oleifera* after two weeks from seed sowing date.

Shade level	**Germination variables**		
	Days for 50% Germination	Germination Rate (days)	Germination percent %
High (80%)	6.05 B	6.88 B	81 B
Medium (50%)	6.08 B	7.05 B	96 A
No (0%)	8.65 A	10.15 A	90 A
P- value	0.0001	0.0001	0.0012

Means with the same letter in the same column are not significantly different at P =0.05 according to Duncan's Multiple Range Test.



(A) High shade (80%)



(B) Medium shade (50%)

(C) No shade (0%)

Figure 5: Effect of three shade levels on germination of *Moringa oleifera* after two weeks from seed sowing.

4.2 Effect of shade on seedling growth

After two weeks from seed sowing and at each of the four harvests the seedlings growth variables were significantly affected by shade level (p=0.0001). The means of the studied growth variables showed significant differences among shade level (Table 2, 3, 4, 5, 6 and 7; Figure 2).

4.2.1 Shoot length

The effect of shade on shoot length on each of the four harvest was highly significant (P= 0.0001). In the first harvest, the high shade achieved the highest shoot length followed by the medium shade (Table 2, Figure 3). However, in the third and fourth harvests; medium shade had significantly the highest shoot length and no shade resulted in significantly the lowest shoot length in all harvests.

In harvest 3, the high shade gave succulent and tender shoot, while the medium and no shades gave erect and strong shoot (Table 3, Figure 4).

4.2.2 Root length

The effect of shade on root length was highly significant (P< 0.0006). Generally, the medium shade resulted in the longest root in the four harvests (Table 4, figure 4).

High shade displayed significantly the lowest root length in all harvests. Also, the high gave small and weak root, while the medium and no shade gave tuberous and swollen roots (Figure 3).

4.2.3 Shoot dry mass

The effect of shade on shoot dry mass was highly significant (P = 0.0001). There were significant differences among most of shade levels in the four harvests (Table 5). The medium shade showed significantly the highest shoot dry mass, while high shade showed significantly the lowest one.

4.2.4 Root dry mass

The effect of shade on Root dry mass was highly significant (P = 0.0001). There were significant differences among the shade levels in the four harvests. The medium shade had the highest Root dry mass, while the high shade had the lowest Root dry mass (Table 6).

4.2.5 Total dry mass

The effect of shade on total dry mass was highly significant (P = 0.0001). There were significant differences among the shade levels in the four harvests (Table 7). The medium shade had the highest total dry mass, while the high shade had the lowest total dry mass.

Table 2: Effect of three shade levels on shoot length (cm) of *Moringa oleifera* on four subsequent seedling harvests.

Shade level	**Shoot length (cm)**			
	Harvest (months)			
	1	2	3	4
High (80%)	32.1 A	49.0 A	58.5 B	52.6 B
Medium (50%)	28.1 B	52.3 A	66.3 A	87.5 A
NO (0%)	10.0 C	11.0 B	19.4 C	55.4 B
P-value	0.0001	0.0001	0.0001	0.0001

Means with the same letter in the same column are not significantly different at P=0.05 according to Duncan's Multiple Range Test.

(A) High shade (80%)

(B) Medium shade (50%)

(C) No shade

Figure 6: Effect of three shade levels on seedlings growth of *Moringa oleifera* after 12 weeks from seed sowing in the third harvest

Table 3: Effect of three shade levels on the stem resistance to cut of *Moringa oleifera* seedling growth after 12 weeks from seed sowing date.

A) Type of seedling growth

Type	High shade (80%)	Medium shade (50%)	No shade (0%)
Soft	100%	10%	30%
Hard	0%	90%	70%

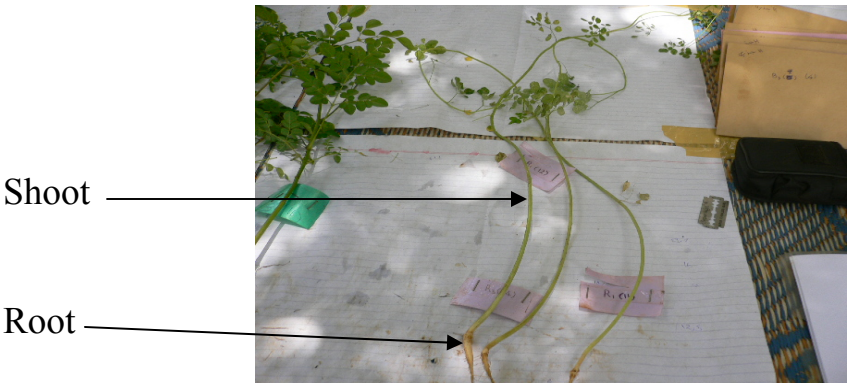
B) Stem resistance to cut

Cutting	High shade (80%)	Medium shade (50%)	No shade (0%)
Easy	75%	0%	0%
Hard	25%	15%	55%
very hard	0%	85%	45%

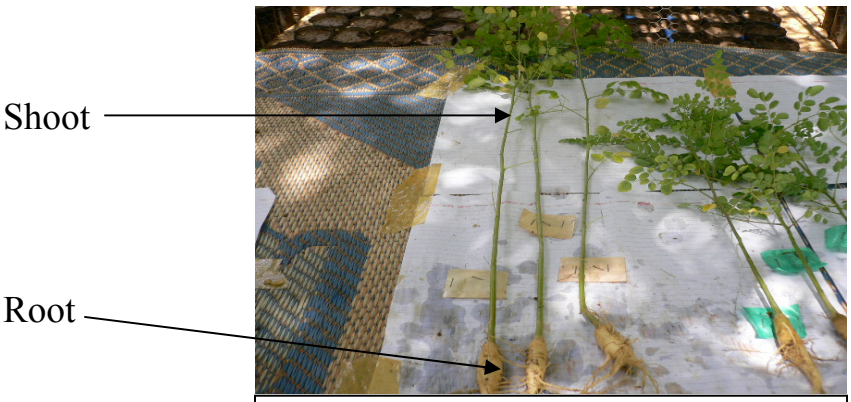
Table 4: Effect of three shade levels on root length of *Moringa oleifera* on four subsequent seedling harvests.

Shade level	**Root length (cm)**			
	Harvest (months)			
	1	2	3	4
High (80%)	5.6 B	6.5 C	6.8 B	6.9 B
Medium (50%)	8.2 A	12.3 A	15.1 A	18.9 A
No (0%)	7.8 A	8.1 B	14.4 A	20.9 A
P-value	0.0006	0.0001	0.0001	0.0001

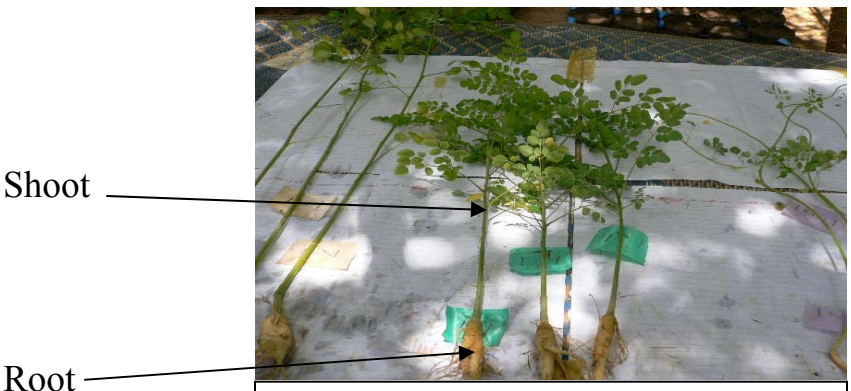
Means with the same letter in the same column are not significantly different at p=0.05 according to Duncan's Multiple Range Test.



(A) High shade (80%)



(B) Medium shade (50%)



(C) No shade (0%)

Figure 7: Effect of three shade levels on shoot and root lengths of *Moringa oleifera* after 12 weeks from seed sowing (third harvest).

Table (5): Effect of three shade levels on shoot dry mass of *Moringa oleifera* on four subsequent seedling harvests.

Shade level	**Shoot dry mass (g)**			
	Harvest (months)			
	1	2	3	4
High (80%)	0.11 C	0.3 B	0.3 C	0.3 B
Medium (50%)	0.25 A	1.1 A	1.5 A	4.2 A
NO (0%)	0.16 B	0.4 B	0.8 B	4.2 A
P-value	0.0001	0.0001	0.0001	0.0001

Means with the same letter in the same column are not significantly different at p=0.05 according to Duncan's Multiple Range Test.

Table 6: Effect of three shade levels on root dry mass of *Moringa oleifera* on four subsequent seedling harvests.

Shade level	**Root dry mass (g)**			
	Harvest (months)			
	1	2	3	4
High (80%)	0.04 C	0.02 C	0.03 C	0.03 B
Medium (50%)	0.05 B	0.9 A	1.9 A	6.1 A
NO (0%)	0.06 A	0.22 B	1.0 B	6.3 A
P-value	0.0001	0.0001	0.0001	0.0001

Means with the same letter in the same column are not significantly different at p=0.05 according to Duncan's Multiple Range Test.

Table 7: Effect of three shade levels on total dry mass of Moringa oleifera on four subsequent seedling harvests

Shade level	**Total dry mass (g)**			
	Harvest (months)			
	1	2	3	4
High (80%)	0.04 C	0.3 C	0.3 C	0.3 B
Medium (50%)	0.3 A	2.0 A	3.4 A	10.3 A
No (0%)	0.2 B	0.6 B	1.7 B	10.5 A
P-value	0.0001	0.0001	0.0001	0.0001

Means with the same letter in the same column are not significantly different at p = 0.05 according to Duncan's Multiple Range Test.

4.2.6 Root to shoot dry mass ratio

The effect of shade on root dry mass/ shoot dry mass ratio was highly significant in the four harvests ($P \leq 0.002$). There were significant differences among the shade levels in the four harvests (Table 8). The medium and no shade had the highest ratio. However, in third and fourth harvests, medium and no shades displayed significantly the highest root/shoot ratio; and High shade displayed the lowest root/shoot ratio.

4.3 Effect of Shade on Stem Wood Anatomy

The results showed highly significant differences among the shade levels ($p=0.0001$) in the anatomical variables. Differences between harvests (seedling age) was significant for vessel volume fraction ($p=0.001$), horizontal vessel diameter ($p=0.01$) and vertical vessel diameter ($p=0.03$). However, the interaction of harvest shade was highly significant for all of the variables. The means and results of the Duncan's Multiple Range Test for the difference among shade levels at each harvest are shown in Tables 9, 10, 11, 12 and 13; figure 5.

4.3.1 Vessel volume fraction

The effect of shade on vessel volume fraction was highly significant ($P = 0.0001$). Generally there is a decrease in the vessel volume fraction with increasing shade (Table 9). High shade had significantly the lowest values in all harvests. The difference

between no and medium shade was only significant in the third harvest.

4.3.2 Horizontal vessel diameter

The effect of shade on horizontal vessel diameter was highly significant (P = 0.0001). There was a decrease in the horizontal vessel diameter with increasing shade (Table 10). High shade had significantly the lowest value in all harvests. The difference between no and medium shade was significant in second and third harvests.

4.3.3 Vertical vessel diameter

The effect of shade level on vertical vessel diameter was highly significant (P = 0.0001). Also, vertical vessel diameter decrease with shade in all harvests (Table 11). The no shade level had significantly the highest values in all harvest. The difference became significant between medium and high shade in the fourth harvest.

4.3.4 Number of vessel

The effect of shade on number of vessel was highly significant (P = 0.0001). Generally, there was a decrease in vessel number with increasing shade (Table 12). High shade had the lowest values in all harvests. The difference between no and medium shade was significant in the first and second harvests.

4.3.5 Volume fraction of lignified cells

The effect of shade on volume fraction of lignified cells was highly significant (P = 0.0001). Generally there is a decrease in the volume fraction of lignified cells with increasing shade (Table 13). High shade had significantly the lowest value in all harvests. The differences between no and medium shade were significant in all harvests except in the second one.

Table 8: Effect of three shade levels on root dry mass/ shoot dry mass ratio of *Moringa oleifera* on four subsequent seedling harvests.

Shade level	**Root dry mass / shoot dry mass ratio**			
	Harvest (months)			
	1	2	3	4
High (80%)	0.31 B	0.08 C	0.13 B	0.10 B
Medium (50%)	0.20 C	0.90 A	1.94 A	1.56 A
No (0%)	0.43 A	0.60 B	1.22 A	1.78 A
P-value	.0001	.0001	.0020	.0001

Means with the same letter in the same column are not significantly different at 0.05 according to Duncan's Multiple Range Test.

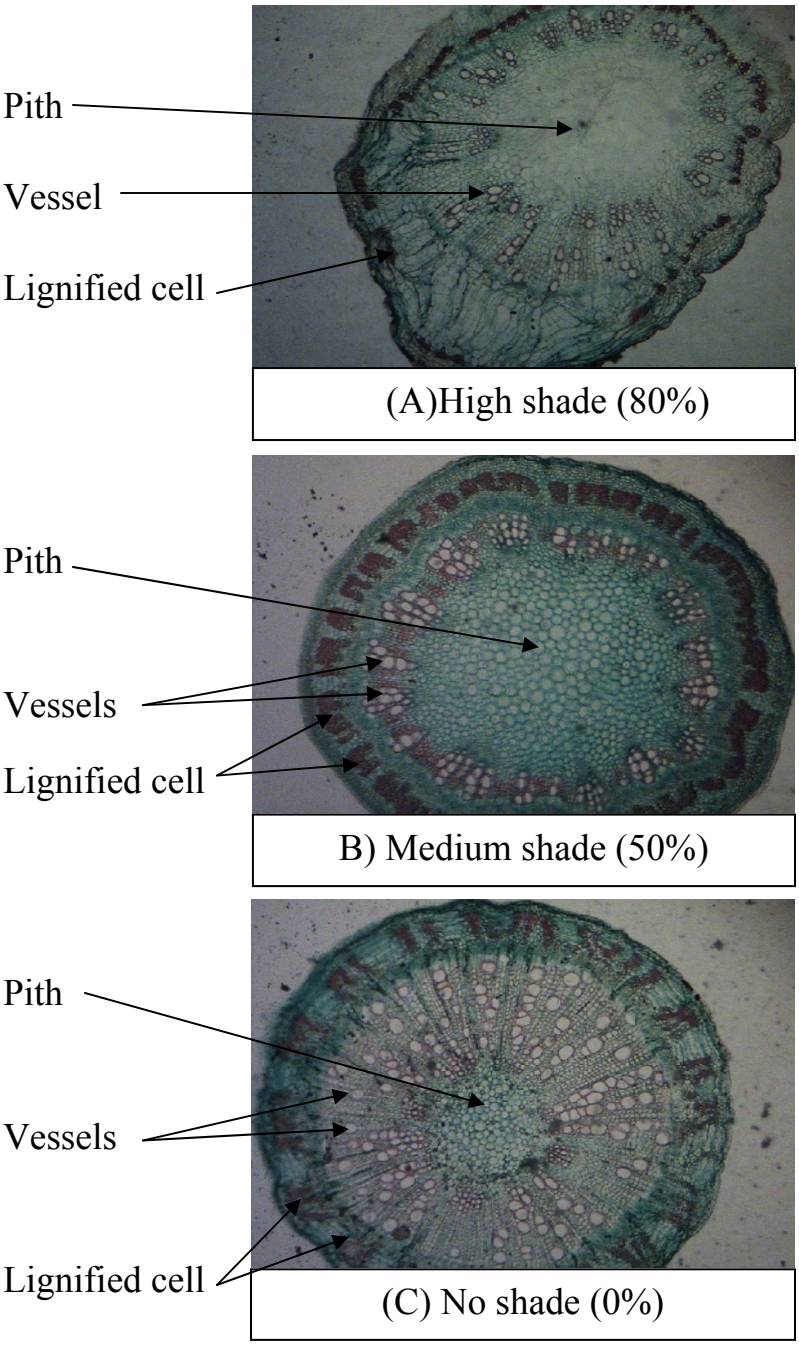


Figure 8: Effect of three shade levels on number of vessel and lignified cells of *Moringa oleifera*, after 12 weeks from seed sowing (third harvest).

Table 9: Effect of three shade levels on vessel volume fraction of *Moringa oleifera* on four subsequent seedling harvests.

Shade level	**Vessel volume fraction**			
	Harvest (months)			
	1	2	3	4
High (80%)	6.563 B	6.597 B	6.563 C	8.125 B
Medium (50%)	13.438 A	15.938 A	11.563 B	20.625 A
No (0%)	15.938 A	17.813 A	21.563 A	24.063 A
P-value	0.0009	0.0001	0.0001	0.0001

Means with the same letter in the same column are not significantly different at p = 0.05 according to Duncan's Multiple Range Test.

Table 10: Effect of three shade levels on horizontal Vessel diameter of *Moringa oleifera* on four subsequent seedling harvests.

Shade level	**Horizontal vessel diameter (mm)**			
	Harvest (months)			
	1	2	3	4
High (80%)	8.958 B	10.088 B	10.833 C	11.458 B
Medium (50%)	17.708 A	14.583 B	17.500 B	24.167 A
NO (0%)	21.458 A	21.875 A	25.000 A	24.583 A
P-value	0.0001	0.0001	0.0001	0.0001

Means with the same letter in the same column are not significantly different at p = 0.05 according to Duncan's Multiple Range Test.

Table 11: Effect of three shade levels on vertical vessel diameter of *Moringa oleifera* on four subsequent seedling harvests

Shade level	**Vertical vessel diameter (mm)**			
	Harvest (months)			
	1	2	3	4
High (80%)	a 9.792 B	13.377 B	14.375 B	12.917 C
Medium (50%)	b 14.792 B	14.583 B	16.042 B	21.667 B
No (0%)	a 22.292 A	23.750 A	24.375 A	28.542 A
P-value	0.0001	0.0264	0.0001	0.0001

Means with the same letter in the same column are not significantly different at p =0.05 according to Duncan's Multiple Range Test.

Table 12: Effect of three shade levels on number of vessel of *Moringa oleifera* on four subsequent seedling harvests.

Shade level	**Number of vessel per /mm2.**			
	Harvest (months)			
	1	2	3	4
High (80%)	7.600 C	9.000 B	11.150 B	10.250 B
Medium (50%)	12.600 B	9.050 B	13.300 AB	16.350 A
No (0%)	21.550 A	21.400 A	15.800 A	16.800 A
P-value	0.0001	0.0001	0.0127	0.0005

Means with the same letter in the same column are not significantly different at 0.05 according to Duncan's Multiple Range Test.

Table 13: Effect of three shade levels on volume fraction of lignified cells of *Moringa oleifera* on four subsequent seedling harvests.

Shade level	**Volume fraction of lignified cells**			
	Harvest (months)			
	1	2	3	4
High (80%)	24.063 C	9.649 C	14.688 C	12.813 C
Medium (50%)	35.313 B	67.188 A	45.625 B	51.875 B
No (0%)	24.063 A	39.063 B	53.438 A	59.688 A
P-value	0.0001	0.0001	0.0001	0.0001

Means with the same letter in the same column are not significantly different at 0.05 according to Duncan's Multiple Range Test.

CHAPTER FIVE

DISCUSSION

The results confirmed the hypothesis that shade level (high shade 80%, medium shade 50% and no shade 0%) had significant effect on the germination, seedlings growth and stem wood anatomy.

5.1 Germination

The high speed of germination of *M. oleifera* in high and medium shades (Table 1), may be due to suitable temperature, light intensity, moisture and aeration for speed of germination, also may be due to less evaporation of water from the soil under high and medium shade similar to that recorded by Paulo, (2006) and Jahn *et al*., (1986). The highest germination percentages were recorded in seeds grown under medium and no shade than seeds grown under high shade ($p=0.0012$). This may be due to less evaporation of water from the soil and suitable light conditions for seeds germination. These results are in agreement with that recorded by Jahn *et al.* (1986), who stated that the optimum light condition for germination of all Moringa species was half shade. Also, Mohammed (1999) indicated that germination of *Capparis dicidua* seeds was higher in shaded than in partially shaded conditions.

The low germination percentage of seeds under high shade level obtained in this study may be due to the effect of low soil temperature or perhaps low light intensity or both of them. These results are similar to the findings drawn by Bonner, *et al*. (1994).

Generally, the result indicates that germination of seeds of *M. oleifera* can occur with high percent under all shade intensities. This means the seeds of *M. oleifera* can germinate over a wide range of temperature and few light intensity, and they have adequate resistance to high temperature as noted by Dishna (2000).

5.2 Shoot length and root length

The result of the shoot length of *M. oleifera* seedlings (Table 2; Figure 4) were similar to that concluded by Jahn *et al*. (1986) and GFU (2008), who mentioned that the growth of *M. oleifera* seedlings is much affected by light conditions, particularly during the hot periods of the year. Early moving of tender seedlings to full light, combined with irregular watering, can thus have disastrous consequences. Also, the result showed that medium shade gave erect and hard seedlings but, high shade gave soft and leaning seedlings (Table 3, Figure 4). This may point to that light intensity was not enough in high shade compared with medium shade. That is similar with the findings of Ballal, (1996) who reported that the use of 50% light intensity in the form of overhead shade significant increased initial height of *Acacia senegal* seedlings.

Also, the longest root was in medium shaded seedlings compared with no and high shades (Table 4 and Figure 4) this might be attributed to the explanation given by Mohammed (1999), who mentioned that the plants with partially shaded condition receive more light which is essential for photosynthesis and then partitioning

of carbohydrates is more in partially shaded plants and this is also dependent on photoperiod and the genetics of the plant.

5.3 Dry mass accumulation

The highest accumulation in medium shade (Table 5, 6 and 7) may be attributed to the microclimatic condition around the seedlings (e.g. threshold light intensity, moderate temperature, high humidity etc.), which were favorable for the growth of seedlings. This result is in agreement with the findings of Jahn *et al*. (1986). Similar results of seedling growth in two tree species, *Gordonia acuminata* and *Cornus controversa,* under relatively dense canopy cover (under shading) have been reported by Cornelissen (1992 and 1993). Bazzaz and Miao, (1993) also reported better seedlings growth of a late successional red oak in low than high light. However, Khan and Shanker (2001) reported better growth of seedlings of *Quercus semiserrata* in medium light condition.

Root to shoot ratio of *M. oleifera* seedlings displayed highest value in no and medium shade, compared with seedlings in high shade (Table 8). The above result may be due to that seedlings grown in medium and no shade might have suffered from water limitation due to higher soil temperature and hence might have caused reduction in the above growth parameters (length and mass of shoot). On the other hand root systems development may be slightly better in the no and medium shade. These results are in agreement with the findings of Mohamad (1986).

Also, the lowest root/shoot ratio in high shade may be due to seedlings growth under limited light conditions; the mass of roots is reduced more than the mass of the shoot, which leads to a decrease in the root/shoot ratio. These results are in agreement with the findings of Pellerin and Demotes-Mainard (1992).

5.4 Stem wood anatomy

The effects of the three shade levels on stem wood anatomy variables shown in Tables 9, 10, 11, 12 and 13 and Figure 4, indicates that vessel volume fraction, horizontal and vertical vessel diameters, number of vessels and volume fraction of lignified cells decreased significantly with increased shade level.

These results indicate that the conductive system was more developed in the seedlings growing under suitable shade level; this is in line with the needs of seedling growth. They indicate that the seedlings growing under shade levels may need a more effective conductive system to cope with their more successive growth needs. The increase in dry mass accumulation may be resulted in the increased photosynthesis, which was associated with the increase in shade intensities. These results are in agreement with the findings of Rury and Dichison (1984).

The decrease in volume fraction of lignified cells with increased shade the result is similar to what was found by Doley (1978), who concluded that the rate of cambial cell division in *Eucalyptus grandis* was approximately 50% greater in the light than in the low light treatment.

CHAPTER SIX

CONCLUSIONS and RECOMMENDATIONS

6.1 Conclusions:

Shade levels had significant effect on:

- Germination.
- Seedlings growth.
- Stem wood anatomy.

1. Germination of *M. oleifera* can occur with high percent under all shade levels.
2. The study indicated that seedling of *M.oleifera* under medium shade level produced significantly the highest biomass accumulation and partition.
3. The study indicated that seedlings of *M. oleifera* were affected by shade level; high shade (80%) produced succulent and tender shoot with small and weak root, while the medium (50%) and no (0%) shades produces erect and strong shoot with tuberous and swollen root.
4. The study indicated that some of anatomical characteristics (vessel volume fraction; horizontal and vertical vessel diameters; and number of vessel; and volume fraction of lignified cells) on stem of *M. oleifera* seedlings were decreased significantly with the increase in shade level.
5. The study revealed the high adaptability of *M. oleifera* to arid and semi arid conditions where every climate factor is inconsistence.

6.2 Recommendations

It is recommended to grow *Moringa oleifera* seedlings under medium shade level (50%) to enhance seedlings growth in the nursery.

References

Akachuku, A. E. and Burley, J. (1979). Variation of anatomy Gmelina arborea Roxb. in Nigerian plantations. AIWA Bull. 4: 94-99.

Amaglo, N. (2006). How to produce moringa leaves efficiently?.
Ghana.www.moringanews.org/doc/GB/Groups/Group_2_Newton_text_GB.

Baas, P. (1973). The wood anatomy range in Ilex (Aquifoliaceae) and its ecological phylogenetic significance. Blumea 21: 193-258.

Baas, P. 1986. Ecological Patterns in xylem anatomy. In: J.J. Givnish (Ed.), The economy of plant form and function. Cambridge University Press, Cambridge, UK. 327-349.

Baas, P. and Schweingruber F.H. (1987). Ecological trends in the wood anatomy of trees, shrubs and climbers from Europe. IAWA Bulletin n. s. 8: 245-274.

Ballal, M.E. (1996). Effect of some nursery practices on quality and field performance of Acacia Senegal seedlings in western Sudan. U.K.J Agric. Sci. 4(2):128- 138.

Bazzaz, F.A. and Miao.S.L. (1993). Successional status, seed size and responses of tree seedlings to CO2, light and nutrients. Ecology 74: 104-112.

Bissing, D.R. (1976). The effect of cultivation on the expression of anatomical features of the wood of selected dicotyledons. Ph.D. Thesis. Claremont Graduate School. Claremont, California. 117 pp.

Bonner, F.T.; Vozzo, J.A.; Elam, W. W. and Land, SB. Jr. (1994). Tree seed technology training course. Instructor's manual. Gen. Tec h. Rep. SO-106. New Orleans: USDA Forest Service, Southern Forest Experiment Station. 160p.

Bonner, F. T. and Vozzo, J. A. (1987). Seed biology and technology of Quercus. Gen. Tech. Rep. SO-66. New Orleans: USDA Forest Service, Southern Forest Experiment Station. 21 p.

Bonner, F. T. (1968). Water uptake and germination of red oak acorns. Botanical Gazzette 129: 83–85.

Bonner, F. T. (1984). Glossary of seed germination terms for tree seed workers. Gen. Tech. Rep. SO-49. New Orleans: USDA Forest Service, Southern Forest Experiment Station. 4p.

Carlquist, S. (1966). Wood anatomy of compositae: A summary, with comments on factors conrolling wood evolution. Aliso 6 (2): 25-44.

Carlquist, S. (1984). Vessel grouping dicotyledons woods : significance and relationship to imperforate tracheary elements. Aliso 10: 505-525.

Carlquist, S. (1987). Diagonal and tangential vessel aggregations in wood: Function and relation-ship to vasicentric tracheids. Aliso 11: 451-462.

Carlquist, S. (1988). Comparative wood anatomy, systematic, ecological and evolutionary aspects of Dicotyledon Wood.

Carlquist, S. and Hoekman. (1985). Observations on functional wood histology of vines and banas: vessel diamorphism, tracheid, vasicentric tracheids, narrow vessels and parenchyma. Aliso.11:139-157.

Core, H.A.; Cote,W.A. and Day A.C. (1976). Wood Structure and Identification, Syracuse University Press. pp 168

Cornelissen, J.H.C. (1992). Seasonal and year to year variation in performance of Gordonia acuminate seedlings in different light environments. Canadian Journal of Botany 70: 2405-2414.

Cornelissen, J.H.C. (1993). Seedling growth and morphology of the deciduous tree Cornus controversa in simulated forest gap light environments in subtropical China. Plant Species Biology 8: 21-27.

Dishna, S. (2000). Water Clarification using Moringa oleifera.Gate Information Service, schborn, Germany. http://www.gtz.de/gate/gateid.afp.

Doley, D. (1978). Effect of Shade on Xylem Development in Seedlings of Eucalyptus Grandis Hill Ex. Maiden. Botany Department, University of Queensland St Lucia,Queenland 4067, Australia. New Phytologist, Vol. 82, No. 2 (Mar., 1979), pp. 545-555

Elamin, H.M.(1981). Trees and shrubs of the Sudan. Ph.D. Thesis Faculty of Science, University of Khartoum. Sudan.

Elias, H. (1976). How stereology come about. Proceedings of the forth international congress for stereology. Edited by Ervin E. Underwood. Published by U.S Government printing office Washington.

Fahey, J. W. (2005). *Moringa oleifera*: A review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part1. Trees for Life Journal, 1:5.

February, E.C. (1993). Sensitivity of xylem vessel size and frequency to rainfall and temperature: implications for palaeontologia Africana 30: 91 -95.

Fielding, J.M. and Brown, A.G. (1960). Variation in the density of the wood Monetary pine from tree to tree. Forestry and timber Bureau leaflet (77): 17.

Gary, W. K. (1988). Professor, Extension environmental horticulturalist, north Florida Research and Education Center

Quiy; Cooperative Extension service, Institute of food and agricultural Science, University of Florida, Gainesville, 32611.

GFU (2007). Global Facilitation Unit for Underutilized Species, Via dei Tre Denari, 472/a 00057 Maccarese, Rome, Italy.

Website: www. underutilized- Species@cgiar.org.

Gibreel, H. H. (2008). A taxonomic study of El Nour natural forest reserve, Blue Nile State, Sudan, M.Sc thesis, University of Khartoum.

Gibson, A.C. (1973). Comparative anatomy of secondary xylem in Cactoideae (Cactaceeae). Biotropica 5: 29-65.

Hartwell, J.L. (1971). Plants used against cancer. A survey. Lloydia 30–34.

Haygreen, J.G. and J.L. Bowyer (1980). Forest products and science. Lowa State University Press, pp 500.

Hébert, Y.; Guingo, E. and Loudet, O. (2001). The response of root/shoot partitioning and root morphology to light reduction in Maize Genotypes. Crop Physiology and Metabolism|. Crop Science,41:363-371 Crop Science Society of America.

Ibrahim, B. B. (2007). Effect of seed resource and irrigation regime on stem wood anatomy of *Acacia senegal* seedlings, Sudan, M.Sc thesis, University of Khartoum.

Ifju, G. (1983). Quantitative wood anatomy certain geometrical statistical relationships. Wood and Fiber Science. 15: (4), pp 326-337.

Jahn, S. A. A.; Musnad, H. A. and Burgstaller, H. (1986). The tree that purifies water: Cultivating multipurpose Moringaceae in the Sudan. Unasylva, 38, pp 23-28.

Jane, F.W.; Wilson, K. and White, D. J. B. (1970). The Structure of Wood. London: Adam and Charles Black. pp108.

Kasperbauer, M. J. and Hunt, P. G. (1992). Root size and shoot⁄ root as influenced by light environment of the shoot. USDA-ARS, Coastal Plains Soil and Water Conservation Research Center Florence, U.S.A. Journal of Plant Nutrition, 15(6and7) 685-697.

Kennedy, R.W. (1961). Variation and periodicity of summer wood in some second-growth. Doglas- fir. Tappi 44 (3): 161-165.

Khalil, M.A. (1985). Genetic of wood characters of Blach Spruce Picea mariana (mill). Silva Genetica 34 (6): 221-229.

Khan, M. L. and Shanker, U. (2001). Effect of seed weight, light regime and substratum microsite on germination and seedling growth of Quercus semiserrata Roxb. Tropical Ecology 42: 117-125.

Kotowski, F. (1926). Temperature relations to germination of vegetable seed. Proceedings of the American Society for Horticultural Science 23:176-184. [Links]

Kramer, P. J. and Kozlowski, T. T. (1979). Physiology of woody plants. New York: Academic Press. 811 p.

Kukachka, B. F. (1978). Wood Anatomy of the Neotropical sapotaceae. I. Bumelia. US Forest Products Research Laboratory. FPL 325, pp 1-9.

Levitt, J. (1972). Responses of Plants to Environmental Stresses. Academic Press, New York. pp. 697.

Lorans, P.R. (1964). The Formation of Wood in Forest Tree, Some Indirect Effect of Environment on Wood Formation. Academic Press. New York. London.

Mahjoub, A. A. (2001). Variation in wood density and shrinkage of twelve species from Kordofan State. M.Sc. Thesis. University of Khartoum, Sudan.

Maynard, B. K. and Bassuk, N.L. (1996). Effect of stock plant etiolation, shading, banding, and shoot development on

histology and cutting propagation of *Carpinus betulus* L. fastigiata. J. AMER. Soc. HORT. SCI. 121(5):853-860.

Medina, E. (1995). Diversity of life forms in neotropical dry forests. In: Bullock SH. Mooney HA, Medina E, eds. Seasonally dry tropical forests. Cambridge: Cambridge University Press, 221-242.

Michener, D. C. (1983). Wood and leaf anatomy of Keckiella (scrophulariaceae): Ecological Considerations. 10 (1): 39-57.

Miller, A. A.; Duysen, M. E. and Wilkinson, G. E. (1975). Internal water balance of barley under soil moisture stress. Plant physiology, Washington, V. 43, p1-17.

Mohamad, A. B. (1986). Light requirements of *Shorea materialis* seedlings. Forest Research Institute Malaysia (FRIM), Kepong, Selangor, Malaysia. Pertanika 9(3), 285 - 289

Mohammed, M. E. (1999). Studies on seed propagation and chemical Composition of Capparis deciduas (Forsk) Edgew (Tundob), Sudan, M.Sc thesis, University of Khartoum.

Nasroun, T.H. and Elzaki, O.T. (1987). The relationship beween anatomical structure and mechanical properties of wood. Sudan Silva 6 (2) 88-99

Nasroun, T. H. (1978). Micro-structural Characteristics of Seven Tropical Hardwoods and their Relationship to Paper Making Properties. Ph.D. Thesis. Virginia Poly-Technical Institute and State University.

Nasroun, T. H. (1979). Home grown timber properties that are required for engineering purposes. In: O.M.E Fageiri and H.O. Abd El Nour (Eds). Proceedings of the Symposium on the Use of Home Grown Wood in Building. Building and Road Research Institute. Khartoum. Sudan. 113-121.

Olson, M. E. and Carlquist, S. (2000). Stem and root anatomical correlations with life form diversity, ecology, and systematics in Moringa (Moringaceae). Botanical-Journal of the Linnean Society (2001), 135: 315-348. With 111 figures doi:10.100§/bojl.2000.0427? www.idealibrary.com

Palada, M. C. and Chang, L. C. (2003). Suggested Cultural Practices for Moringa. Asian Vegetable Research and Development Center. Taiwan. www: http://www.avrdc.org.

Paulo, S. (2006). How and why to measure the germination process? Rev. bras. Bot. vol.29 no.1

Pellerin, S. and Demotes-Mainard, S. (1992). Effect of competition for light between plants on the root shoot ratio and the number of adventitious roots of maize. p. 65–68. In L. Kutschera et al. (ed.)

Rury, P. and Dickinson, W. (1984). Structural correlations among woody leaves and plant habit. In: R.A White and W. Dickinson (Eds) Contemporary problems in plant anatomy. Orlando, Florida: Academic press, Inc.

Simon EW. (1984). Early events in germination. In: Murray DR, ed. Seed physiology. Volume 2, Germination and reserve mobilization. Orlando, FL: Academic Press: 77–115.

Underwood, E.E. (1970). Quantitative Stereology. Addison-Wesley Publication Co. Reading, MA. pp 274.

Van der Walt, J.J.A.; Werker, E. and Fahn, A. (1988). Wood anatomy of the Plargonium (Geraniaceae). IAWA Buletin 10: 201-207.

Verma, S. C.; Banerji, R.; Misra, G. and Nigam S. k. (1976). Nutritional value of *Moringa*, via *Moringa oleifera*; medicinal and socio–economic uses.

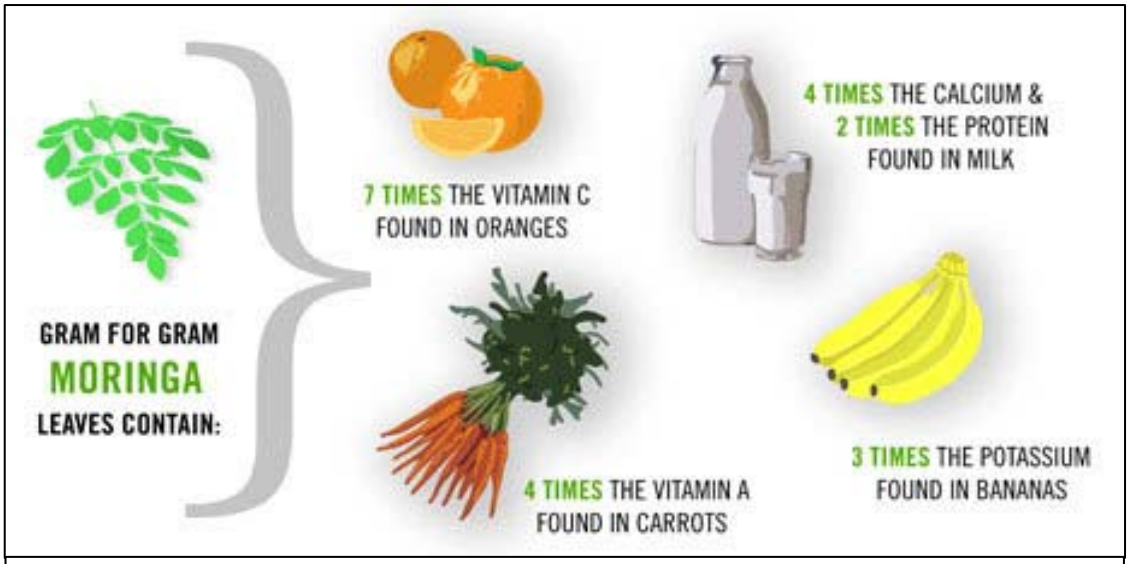
Vertucci, C. W. (1989). The kinetics of seed imbibitions: controlling factors and relevance to seedling vigor. In: Stanwood PC, McDonald MB, eds. Seed moisture. Spec. Publ. 14. Madison, WI: Crop Science of America: 93–115.

Von Madell, H. J. (1986). Trees and shrubs of the Sahel, their characteristics and uses. GTZ , Eschborn, Germany. 525 pp.

Wilkins, A. P. and Papassotiriou, S. (1989). Wood anatomical variation of Acacia melanoxylon in relation to latitude IAWA Bulletin n. s. 10: 201-207.

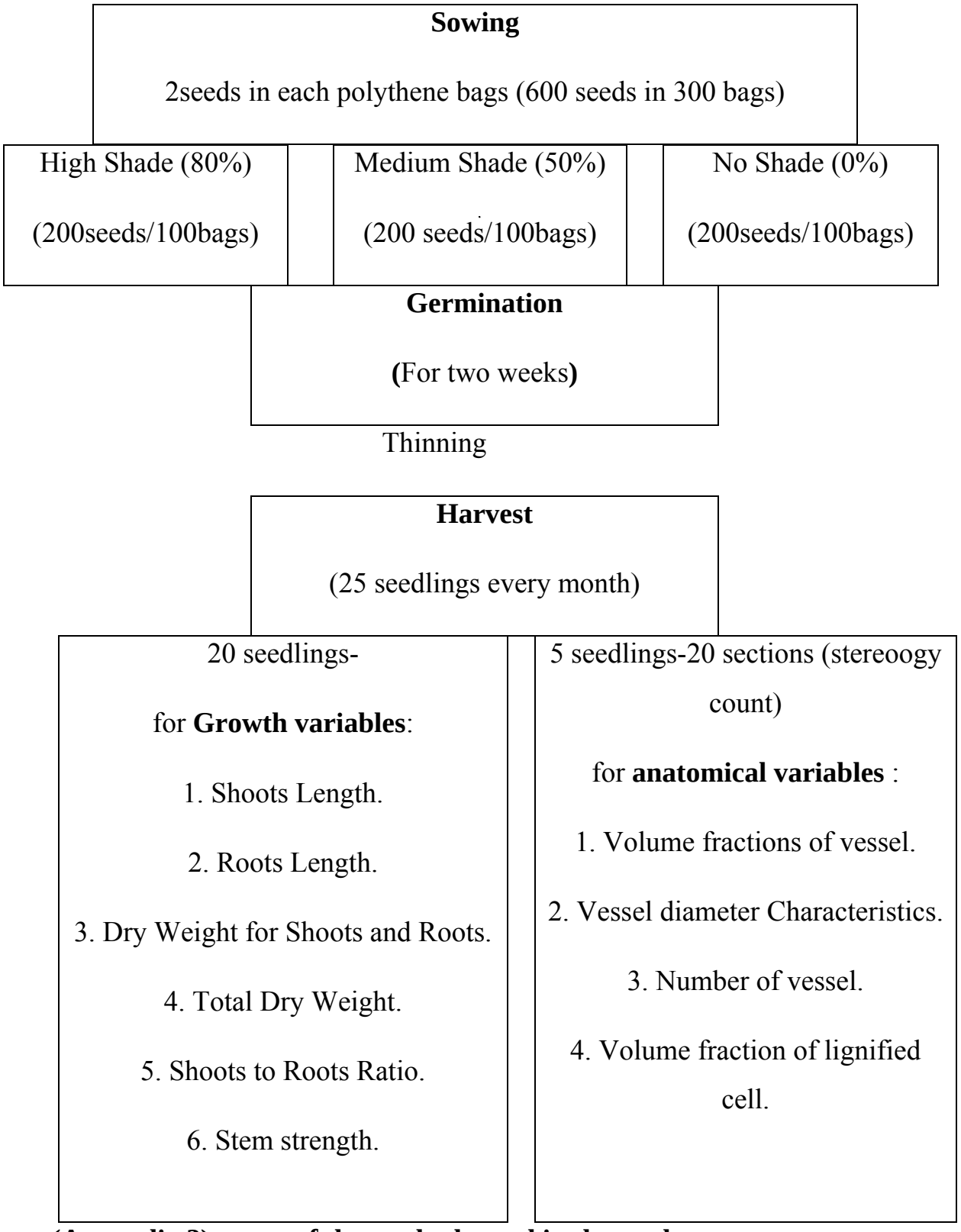
Zhang, X; Deng, L. and Baas, P. (1988). The ecological wood anatomy of the Lilacs(Syringa oblate var. giraldii) on Mount Taibei in Nourth-western China. IAWA Bulletin n. s. 9: 24 30.

Appendixes



Appendix 1: Nutritional value of *Moringa oleifera* leaves (Fahey,

(Appendix 2): The set up used for steer counting including the 'Moticam 1000' digital camera mounted on an 'Olympus CH20' microscope and the computer monitor.



(Appendix 3): steps of the methods used in the study.